REMARKS

Applicants believe no new matter is added by this amendment.

Amendments to the specification.

The amendment to the specification adding the new paragraph at page 1, line 22 indicates the cooperative research effort between Mendel Biotechnology, Inc. and Monsanto Company.

Amendments to the claims.

Claims 1-39 have been canceled. New claims 39-61 are being added.

Support for the amendments to the claims may be found, for example:

for "a CCAAT-binding transcription factor", in priority application 60/125,814, filed March 23, 1999, on page 25 at lines 19-20 ("Family 13. CAAT Binding Protein Family of Transcription Factors", and describing the HAP3 subunit) and in the attached Appendix of sequences (see attached, showing the G482 protein and DNA sequences, the former being truncated by four n-terminal amino acids, filed with this priority application), in priority application 60,166,228 (with the full length sequence) describing the G482, SEQ ID NO: 4 sequence as "equivalent to AtHAP3b"; and in the present application at, for example, page 34, lines 16-18 and at page 40, line 20, describing the G482 sequence as "belong[ing] to the HAP3 class";

for 5X SSC and 50% formamide at 42°C, in priority application 60,166,228, filed 11/17/99, on page 27, lines 9-10 (see attached), and in the present application on page 46, lines 7-9;

for at least two wash steps of 0.2X SSC, 1% SDS at 65°C, in priority application 60,166,228, filed 11/17/99, on page 27, lines 11-13 of priority application 60,166,228 (see attached; describing low stringency washes "prior to high stringency washes"), and in the present application on page 6 at line 25, at page 45 at line 23 (the latter indicates the use of additional wash steps, if necessary), and on page 47, line 8 ("at least two final wash steps");

for 0.1X SSC, 0.1% SDS at 65° C for 30 min for each step, in the present specification at page 40, lines 31-32;

for greater cotyledon expansion than a control plant after growing for three days in the presence of 150 mM NaCl, on page 10, lines 5-8 of the present specification; and

for transformed seed, on page 7, lines 9-17 and on page 84, line 24 through page 85, line 9.

Response to specific items in the Office action

Item 6. Priority

Applicants believe the presently amended claims are supported by the description presented in priority application 60/166,228, filed November 17, 1999 and in priority application 60/125,814, filed

03/23/99. As indicated above, specific claim elements that are included in the present amendment were described in these applications. The attached pages, with pertinent lines presently underlined, indicate where these elements may be found.

Accordingly, Applicants believe the instant and priority applications 60/166,228, filed November 17, 1999. and 60/125,814, filed 03/23/99, disclose the present sequences, transgenic plants, and methods for determining salt stress tolerance.

Item 12. 35 U.S.C. §112, first paragraph, written description

Applicants believe that the rejection under 35 U.S.C. §112, first paragraph, for lack of an adequate written description, has been avoided by the amendment of the claims. Other aspects of this rejection are respectfully traversed.

In the case of the present specification, the skilled artisan would, upon reading the present application, understand that Applicants were in possession of the claimed invention in light of the extensive disclosure of a significant number of sequences included within the scope of the claims, and both the conventional and well-known methods, as well as the methods presently disclosed, of identifying related polypeptides, including CCAAT-binding transcription factors, and testing these sequences in plants. It would thus have been made clear to said skilled artisan that Applicants were in possession of the invention at the time the present application was filed.

Furthermore, contrary to the assertions provided in the Office action, one of skill in the art could readily envision each of the polynucleotide sequences that hybridize to SEQ ID NO: 3 based on the hybridization analysis being claimed. The specification contains an unambiguous description of the structure of any member of the claimed genus by reciting the conditions required for hybridization to a canonical sequence, SEQ ID NO: 3.

The *Written Description Training Materials*, Rev. 1, March 25, 2008, Example 6, provides an example of a specification that discloses a novel cDNA (SEQ ID NO: 1) which encodes a protein that binds to a newly-discovered growth factor (NDG) receptor and stimulates tyrosine kinase activity. The specification includes an example demonstrating that the protein encoded by SEQ ID NO: 1 binds to the NDG receptor and stimulates tyrosine kinase activity. SEQ ID NO: 1 was not placed into a public depository. The specification expressly defines highly stringent hybridization conditions as: at least about 6X SSC and 1% SDS at 65°C, with a first wash for 10 minutes at about 42°C with about 20% (v/v) formamide in 0.1X SSC, and with a subsequent wash with 0.2X SSC and 0.1% SDS at 65°C. It is known in the art that hybridization technique using a known nucleic acid as a probe under highly stringent conditions, such as those set for in the specification, will identify structurally similar nucleic acids. The specification in this example discloses an actual reduction to practice and the complete chemical structure of only one species of the claimed genus of nucleic acids (i.e., SEQ ID NO: 1). Because hybridization under highly stringent

conditions requires a high degree of structural complementarity, nucleic acids that hybridize to the complement of SEQ ID NO: 1 must share many nucleotides in common with SEQ ID NO: 1. Thus, the claimed genus necessarily includes partial structures of SEQ ID NO: 1. The disclosure of SEQ ID NO: 1 combined with the knowledge in the art regarding hybridization would put one in possession of the genus of nucleic acids that would hybridize under stringent conditions to SEQ ID NO: 1.

The application also includes Claim 3: "An isolated nucleic acid that encodes a protein that binds to the NDG receptor and stimulates tyrosine kinase activity, wherein the nucleic acid hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1."

The analysis of claim 3 indicates that, without a recognized correlation between structure and function, those of ordinary skill in the art would not be able to identify without further testing which of those nucleic acids that hybridize to SEQ ID NO: 1 would also encode a polypeptide that binds to NDG receptor and stimulates tyrosine kinase activity.

Similar to the fact pattern in Example 6 of the *Training Materials*, the present application discloses a novel cDNA (SEQ ID NO: 3) that encodes a protein (SEQ ID NO: 4). Unlike Example 6, however, there is indeed a known correlation between structure and function. One skilled in the art would understand that the G482 protein sequence, as well as orthologs of G482 (by definition, an ortholog is a functional, phylogenetically-related sequence), each having a CCAAT-binding domain, would be CCAAT-binding transcription factors with the function of binding to a transcription regulating region of DNA comprising the motif CCAAT and regulation transcription.

The present application also positively distinguishes itself from Example 6 of the *Training Materials* by describing numerous sequences closely related to G482, rather than the "only one species of the claimed genus of nucleic acids". G481, G482, G485 and G3395, SEQ ID NOs: 2, 4, 6, and 74, respectively, were each shown to be more tolerant to salt (page 90 at line 21, page 92 at line 7, page 93 line 24, and page 94 line 27, respectively. These sequences which include three eudicot and one monocot-derived sequences, represent a considerably broad sampling of the plant kingdom, as previously indicated.

In light of these amendments, arguments and experimental observations confirming Applicants' disclosure and claims, Applicants request that the rejection under 35 U.S.C. §112, first paragraph, for lack of written description, be withdrawn.

Item 8. 35 U.S.C. §112, first paragraph, enablement

The rejection under 35 U.S.C. §112, first paragraph, for being non-enabling, is respectfully traversed.

To comply with the enablement requirement, the specification must set forth the information sufficient to allow one of ordinary skill to make and use the invention. This may be accomplished through

various means, including actual reduction to practice, prophetic examples, illustrative examples and/or broad terminology and methods, provided the disclosure corresponds with the scope of the claims, and provided there is no reason to doubt the objective truth of the statements relied upon for objective support (*In re Marzocchi*, 169 USPQ 367 (CCPA 1971)).

Methods for identifying related sequences and determining their function, in this case the ability to confer greater salt tolerance to plants, are provided in the specification. Furthermore, , these methods, at the time of the instant filing, were known in the art, and the level of skill in the art was high. Even if a large amount of experimentation is required, said amount is permitted if it is routine.

The methods provided in the specification and known in the art are routine, and the practice of identifying functional species of the claims and testing them in plants is a matter of routine, as Applicants have demonstrated. The present application describes four related CCAAT-binding transcription factors, G481, G482, G485 and G3395, that were each shown to be more tolerant to high salt (page 90 at line 21, page 92 at line 7, page 93 line 24, and page 94 line 27, respectively). Thus, based on this disclosure and the knowledge in the art, Applicants submit that the skilled artisan would not find it unduly burdensome to identify function species of the claims.

In light of these amendments, arguments and experimental observations confirming Applicants' disclosure and claims, Applicants request that the rejection under 35 U.S.C. §112, first paragraph, for lack of enablement, be withdrawn.

Item 9. 35 U.S.C. §102(b)

As indicated above, Applicants believe the presently amended claims are supported by the description presented in priority application 60/166,228, filed November 17, 1999, which is before the filing date of US application 60/196,001 (7 April 2000) cited as a priority document for US patent 6,677,504.

Accordingly, Applicants request that the rejection under 35 U.S.C. §102(e) be withdrawn.

Items 10-11. 35 U.S.C. §103(a)

The rejection under 35 U.S.C. §103(a) is respectfully traversed.

As the present Office action indicates, Edwards et al., (1998) *Plant Physiol.* 117: 1015-1022), page 1021, first column, "the expression of AtHAP3b in leaves from plants … may suggest environmental ***regulation*** of this gene", and "further research is required to understand the regulation of these factors and their role in developmental and environmental ***responses***" (emphasis added). As the Examiner is aware, the fact that a sequence is expressed in response to an general, as is presently the case, or even a specific environmental influence is not an indication that it will confer tolerance to a specific stress when it is overexpressed. Thus skilled in the art understand that 100s of sequences may be up- or down-regulated in response to an environmental influence. Even nine years after Edwards, it was recognized in Swindell et al. (2007) "The biological limitations of transcriptomics in elucidating stress and stress responses." *Heredity* 99:

143–150, that "[c]andidate genes *with a well-supported role in stress-response pathways* provide good prospects for subsequent experimental study" (*emphasis* added; page 149, left column), but "[t]he identification of temperature-related genes [i.e., regulated in response to environmental changes] through microarray analysis represents *only a first step* towards understanding their role in cold- and heat-stress-regulatory pathways"(*emphasis* added; page 149, left column). See also Feder et al. (2005) *J. Evol. Biol.* 18: 901-910; "[p]ublished work to date suggests that mRNA abundance typically provides little information on protein activity and fitness and *cannot substitute for detailed functional and ecological analyses of candidate genes*" (*emphasis* added; Abstract). Thus, even today, the skilled artisan would not be inclined to attempt to overexpress each individual sequence determined to respond to an environmental stress to determine if they can be used to produce *tolerance* to a stress. Indeed, Edwards is silent with respect to whether the liquid culture was hypo-osmotic or hyper-osmotic, and thus is silent with respect to whether HAP3 sequences might produce greater tolerance or sensitivity to an environmental stress. Edwards is silent with respect to how expression in leaves would affect tolerance to salt.

The fact that Edwards et al. recognized that "further research is required to understand the regulation of these factors" indicates that Edwards et al. were aware that they had not provided a completed understanding of the "characteristic of abiotic stress tolerance that would have naturally flown from the use of the AtHAP3b CAAT-box transcription factor[s]" (Office action). In fact, although finding the claimed salt tolerant plants is a matter of routine, Applicants have discovered that lines transformed with G482 and phylogenetically-related sequences do not *necessarily* have increased tolerance to salt stress. See, for example, the specification at page 94, lines 26-27: "[o]ne of the lines of G3395 overexpressors tested was found to be more tolerant to high salt levels", or Table 6, showing that the lines produced with G482 and the two-component supertransformation approach were not more salt tolerant (at least some lines with a direct fusion approach were) whereas G481-overexpressing lines produced with a superactivation approach were more tolerant to salt but direct fusion lines were not (at the time of filing).

In order for an inherent quality to qualify as an element sufficient to anticipate a claimed element, that inherent quality must necessarily and inevitably follow from the teaching. The Federal Circuit carefully distinguished between an accidental anticipation and inherent anticipation in the decision in Schering Corp. v. Geneva Pharms, Inc., 339 F.3d 1373 (2003). Specifically, the court referred to prior case law where the Supreme Court had held that accidental inherency was not sufficient for inherent anticipation: "In Eibel, the Court found no evidence of the claimed subject matter in the prior art. Eibel, 261 U.S. at 66 ('We find no evidence that any pitch of the wire . . . had brought about such a result . . . and . . . if it had done so under unusual conditions, accidental results, not intended and not appreciated, do not constitute anticipation.')." Whereas the Federal Circuit held that, "[i]n the context of accidental anticipation, DCL is not formed accidentally or under unusual conditions when loratadine is ingested. The record shows that DCL *necessarily*

and inevitably forms from loratadine under normal conditions. DCL is a necessary consequence of administering loratadine to patients" (*emphasis* added)." See Schering at 1378. Thus, the fact that the G482 overexpression may produce increased tolerance to salt is not sufficient to anticipate by inherency the present claims to plants.

Accordingly, Applicants request that the rejection under 35 U.S.C. §103(b) be withdrawn.

Items 12-13, Provisional obviousness type double patenting

Applicants note that the MPEP indicates that: "The public should . . . be able to act on the assumption that upon the expiration of the patent it will be free to use not only the invention claimed in the patent but also modifications or variants which *would have been obvious* to those of ordinary skill in the art *at the time the invention was made*, taking into account the skill in the art and prior art other than the invention claimed in the issued patent" (*emphasis* added). The present application, being the earlier filed application with the earlier priority date, would not have been obvious to one of ordinary skill in light of U.S. patent application 11/069,255, since the latter did not yet exist at the time the present invention was made.

The MPEP also indicates that: "If a "provisional" nonstatutory obviousness-type double patenting (ODP) rejection is the only rejection remaining in the earlier filed of the two pending applications, while the later-filed application is rejectable on other grounds, the examiner should withdraw that rejection and permit the earlier-filed application to issue as a patent without a terminal disclaimer." (MPEP 804(I)(B)(1)).

In light of the present amendments and arguments, Applicants believe that all other rejections may be presently overcome, and thus respectfully request that this ground of rejection be held in abeyance until patentable subject matter is defined for the present application.

CONCLUSION

Applicants believe that no additional fee is due with this communication. However, if the USPTO determines that a fee is due, the Commissioner is hereby authorized to charge Mendel Biotechnology, Inc. Deposit Account No. **50-1025.**

Respectfully submitted,
MENDEL BIOTECHNOLOGY, INC.

Date: 15 September 2008

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Attachments:

- 9 pages from priority applications 60/125,814 and 60/166,128
- Feder (2005)
- Swindell (2007)

among the family members. At the compositional level, the family members contain the same putative functional domains, all of which are common among GF14 proteins: potential protein kinase A and kinase C phosphorylation sites, a potential leucine zipper region, and an E-F hand domain, indicative of calcium-binding activity. The family members also share a high degree of homology in regions for which there is no defined putative function. For genes in which it has been examined, gene expression appears to be tissue specific, possibly suggesting specialized functions for the different family members. For AFT1, expression is highest in flower tissue, but is also expressed to varying degrees in root, stem, leaf, and silique tissue (Zhang et al., 1995). For the Chi gene, expression appears to be highest in immature flowers, male-specific and female-specific mature floral tissue, in immature and mature silique tissue, in seed endosperm, and in root epidermal tissue (Daugherty et al., 1996). Although information about GF14 gene expression is accumulating, the exact function(s) of GF14 proteins and the exact role(s) they may play in cellular and physiological processes remains unknown.

Family 13. CAAT Binding Protein Family of Transcription Factors

The regulation of transcription in most eukaryotic genes occurs through the binding of transcription factors to sequence specific binding sites in their promoter regions. Many of the protein binding sites have been conserved throughout evolution and are found in the promoters of diverse eukaryotic organisms. One such site that shows a high degree of conservation is the CCAAT-box element (Gelinas et al., 1985). Proteins that bind to this element have been identified in yeast, mammals and plants. The first proteins identified that bind to this element were identified in yeast and were collectively called the HAP complex (heme activator protein complex) or the CCAAT binding factor (CBF) (Forsburg and Guarente, 1988). The complex is composed of at least four subunits, HAP2, HAP3, HAP4 and HAP5. In yeast, proteins in this complex are

represented by single genes and their function is specific for the activation of genes involved in mitochondrial biogenesis and energy metabolism (Dang et al., 1996) In mammals and plants, analogous members of the complex are represented by small gene families and it is likely that these genes play a more complex role in regulating gene transcription in these organisms. In support of this notion, the CCAAT-box motif is found in the promoters of a variety of plant genes that fail to show any unifying expression pattern. In addition, the expression pattern of many of the HAP-like genes in *Arabidopsis* show developmental regulation. For instance, the ATHAP3a gene is predominately expressed in the flower and silique and the AtHAP3b gene was specifically expressed in leaf, flower and silique (Edwards et al., 1998). Other *Arabidopsis* HAP genes are expressed ubiquitously.

Genetic analysis has determined the function of only one *Arabidopsis* HAP-like gene, *Leafy Cotyledon(LEC1)*. *LEC1* is a HAP3 subunit homolog that accumulates only during seed development. *Arabidopsis* plants carrying a mutation in the LEC1 gene display embryos that are intolerant to desiccation and show defects in seed maturation (Lotan et al., 1998). This is an interesting phenotype that can be rescued if the embryos allowed to grow before the desiccation process occurs during normal seed maturation. (If one wanted to regulate seed maturation and or fertility, this could be one way). The mutant plants also possess trichomes, or epidermal hairs on their cotyledons, a characteristic that is normally restricted to adult tissues like leaves and stems. This phenotype suggests that *LEC1* also has a role in specifying embryonic organ identity. In addition to the mutant analysis, the ectopic expression (unregulated overexpression) of the wild type LEC1 gene induces embryonic programs and embryo development in vegetative cells consistent with its role in coordinating higher plant embryo development. The very specific role of this CCAAT box binding protein this plant developmental process suggests that the CBF complexes in plant are not merely serving a general

transcriptional regulators but are likely to be playing more specific roles in plant growth and development.

Family 14. SPBP Family of Transcription Factors

Flowering is the main form of reproduction in higher plants. Flowering time or the time a plant requires from germination to flowering is an important agricultural trait. The growth of many vegetable and horticultural crops are excluded from certain areas because the time required for them to flower, produce marketable fruit, or seed exceeds the length of the growing season. Flowering time in different plant species is regulated by many developmental and environmental factors and affects crop productivity, both quantitatively and qualitatively. Altering flowering time in plants has represented a serious challenge to traditional plant breeders. The ability to regulate this trait to meet the growers needs for a particular crop would be very valuable and is an area amenable to genetic engineering

In recent years, several genes which regulate flower development have been found, largely through genetic analysis of the model plants species, *Arabidopsis thaliana* and *Antirrhinum majus*. Most of these genes were found to be transcriptional regulators that are responsible for the initiation of a cascade of molecular events leading to the development of a flower. The transition from vegetative growth to reproductive growth is controlled genetically. In particular, the transition to flowering in *Antirrhinum majus*, is thought to be governed by regulators of early genes required for flower development such as the *squamosa* gene product (Klein et al., 1996). Proteins that regulate developmental determinants, such as *squamosa*, are obvious candidates for regulators of the transition to flowering. A study of proteins that potentially regulate floral transition resulted in the identification of a new family of DNA binding proteins (putative transcriptional regulators) called squamosa promoter binding proteins or SBPs. These genes are represented as small gene family in *Arabidopsis* (estimated to have approximately 16 members). There is at least one

>G481 (gf=13) (nid=2398526) [acc=Y13723] 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>P1_G481: fr=+1 (gf=13) (nid=2398526) (acc=Y13723) 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>P2_G481: fr=+2 (gf=13) (nid=2398526) (acc=Y13723) 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>P3_G481: fr=+3 (gf=13) (nid=2398526) (acc=Y13723) 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>M1_G481: fr=- (gf=13) (nid=2398526) (acc=Y13723) 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>M2_G481: fr=-2 (gf=13) (nid=2398526) (acc=Y13723) 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>M3_G481: fr=-3 (gf=13) (nid=2398526) (acc=Y13723) 2398526 gi|2398526|emb|Y13723|ATHAP3A Arabidopsis thaliana mRNA for Hap3a transcription factor

>P1_G482: fr=+1 (gf=13) (nid=2398528) (acc=Y13724) 2398528 gi|2398528|emb|Y13724|ATHAP3B Arabidopsis thaliana mRNA for Hap3b transcription factor

>P2_G482: fr=+2 (gf=13) (nid=2398528) (acc=Y13724) 2398528 gi|2398528|emb|Y13724|ATHAP3B Arabidopsis thaliana mRNA for Hap3b transcription factor

>P3_G482: fr=+3 (gf=13) (nid=2398528) (acc=Y13724) 2398528 gi|2398528|emb|Y13724|ATHAP3B Arabidopsis thaliana mRNA for Hap3b transcription factor

>M1_G482: fr=- (gf=13) (nid=2398528) (acc=Y13724) 2398528 gi|2398528|emb|Y13724|ATHAP3B Arabidopsis thaliana mRNA for Hap3b transcription factor

>M2_G482: fr=-2 (gf=13) (nid=2398528) (acc=Y13724) 2398528 gi|2398528|emb|Y13724|ATHAP3B Arabidopsis thaliana mRNA for Hap3b transcription factor

>M3_G482: fr=-3 (gf=13) (nid=2398528) (acc=Y13724) 2398528 gi|2398528|emb|Y13724|ATHAP3B Arabidopsis thaliana mRNA for Hap3b transcription factor

Summary

Published Function
G482 is equivalent to AtHAP3b which was identified by Edwards et al. (1998) as an EST with homology to the yeast gene HAP3b . Their northern blot data suggests that AtHAP3b is expressed primarily in roots. No other functional information regarding G482 is publicly available.

Mendel Discovery
The function of G482 was analyzed at Mendel through its ectopic overexpression in plants. G482 overexpressors are more tolerant to high NaCl in a germination assay. Further experiments should be done to determine the utility of this degree of NaCl tolerance under field conditions.
RT-PCR analysis of endogenous levels of G482 transcripts indicate that this gene is expressed constitutively in all tissues tested. A cDNA array experiment supports the RT-PCR derived tissue distribution data. G482 is not induced above basal levels in response to any environmental stress treatments tested.

Closely Related Genes
The closest homology in the non-Arabidopsis plant database is within the conserved domain of G482 and therefore no potentially orthologous genes are available in the public domain.

Utility
The potential utilities of this gene include the ability to confer salt tolerance during the germination stage of a crop plant. This would most likely impact survivability and yield. Evaporation of water from the soil surface causes upward water movement and salt accumulation in the upper soil layer, where the seeds are placed. Thus, germination normally takes place at a salt concentration much higher than the mean salt concentration in the whole soil profile.

References
Edwards D, et al. ÿÿMultiple genes encoding the conserved CCAAT-box transcription factor complex are expressed in Arabidopsis. Plant Physiol. 1998 Jul;117(3):1015-22.

Keywords
Salt, Germination
Plasmid ID P47
Cloning Vector pMEN20
Cloning Site SalINotI
Source DNA N97233
Bacterial Strain DH5a
Scientist Jacqueline Heard

Expression

RT-PCR analysis of endogenous levels of G482 transcripts indicate that this gene is expressed constitutively in all tissues tested. A cDNA array experiment confirms this RT-PCR derived tissue distribution data by. G482 is not induced above basal levels in response to any environmental stress treatments tested.

Morphology

Plants overexpressing G482 have a wild-type morphology. Based on kanamycin resistance segregation ratios in the T2 generation, both lines 6 and 12 could each have more than one copy of the transgene inserted in the genome.

Biochemistry

Results from the biochemical analysis of G482 overexpressors suggests that line 12 has higher levels of most seed fatty acids when analyzed by FID. However, NIR data did not show an increase in total seed oil for this line. The FID data is preliminary and will be repeated.

Sequences

TBLASTX versus GenBank non-Arabidopsis plant DNA

AI495007	1.4e-46	[Glycine max] sa89f03.y1 Gm-c1004 Glycine max cDNA clone GENO
X59714	9.2e-45	[Zea mays] Z.mays mRNA for CAAT-box DNA binding protein subun
AI486503	3.3e-42	[Lycopersicon esculentum] EST244824 tomato ovary, TAMU Lycope
AI725612	4.8e-41	[Gossypium hirsutum] BNLGHi12445 Six-day Cotton fiber Gossypi
AW743377	6.1e-33	[Pinus taeda] ST32F09 Pine TriplEx shoot tip library Pinus ta
AA660543	1.7e-16	[Medicago truncatula] 00429 MtRHE Medicago truncatula cDNA 5'
C19737	1.9e-12	[Oryza sativa] C19737 Rice panicle at ripening stage Oryza sa
AT002114	2.9e-11	[Brassica rapa subsp. pekinensis] AT002114 Flower bud cDNA Br
AI967494	0.000100	[Lotus japonicus] Ljirnpest03-196-b3 Ljirnp Lambda HybriZapt
X59057	0.001800	[Antirrhinum majus] A.majus transposable element Tam4 DNA.

BLASTX versus GenBank non-Arabidopsis plant peptide sequences

gi22380	3.4e-46	[Zea mays] CAAT-box DNA binding protein subunit B (NF-YB).
gi115840	3.4e-46	[Zea mays] CCAAT-BINDING TRANSCRIPTION FACTOR SUBUNIT A (CB
gi82091	0.001500	[Lycopersicon esculentum] hydroxyproline-rich glycoprotein
gi170454	0.001500	[Lycopersicon esculentum] cell wall hydroxyproline-rich gly
gi1224726	0.001500	[Lycopersicon esculentum] glycoprotein,Hyp rich.
gi20247	0.001800	[Oryza sativa] glycine-rich cell wall protein.
gi173321	0.001800	[Oryza sativa] glycine-rich cell wall structural protein 1
gi121626	0.001800	[Oryza sativa] GLYCINE-RICH CELL WALL STRUCTURAL PROTEIN 1
gi2293480	0.003500	[Oryza sativa] glycine-rich protein.
gi2267593	0.004000	[Oryza sativa] glycine-rich RNA-binding protein.

DNA sequence used for BLAST

>G482 (gf=13) (nid=4589434,69357,69929) (acc=AB025628) 4589434 gi|4589434|db

```
ATGGGGGATTCCGACAGGGATTCCGGTGGAGGGCAAAACGGGAACAACCAGAACGGACAG
TCCTCCTTGTCTCCAAGAGAGCAAGACAGGTTCTTGCCGATCGCTAACGTCAGCCGGATC
ATGAAGAAGGCCTTGCCCGCCAACGCCAAGATCTCTAAAGATGCCAAAGAGACGATGCAG
GAGTGTGTCTCCGAGTTCATCAGCTTCGTCACCGGAGAAGCATCTGATAAGTGTCAGAAG
GAGAAGAGGAAGACGATCAACGGAGACGATTTGCTCTGGGCTATGACTACTCTAGGTTTT
GAGGATTATGTTGAGCCATTGAAAGTTTACTTGCAGAGGTTTAGGGAGATCGAAGGGGAG
AGGACTGGACTAGGGAGGCCACAGACTGGTGGTGAGGTCGGAGAGCATCAGAAGAGATGCT
GTCGGAGATGGCGGTGGGTTCTACGGTGGTGGTGGTGGGATGCAGTATCACCAACATCAT
CAGTTTCTTCACCAGCAGAACCATATGTATGGAGCCACAGGTGGCGGTAGCGACAGTGGA
GGTGGAGCTGCCTCCGGTAGGACAAGGACTTAA
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Amino Acid Sequence

>G482 Amino Acid Sequence

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MGDSDRDSGGGQNGNNQNGQSSLSPREQDRFLPIANVSRIMKKALPANAKISKDAKETMQ
ECVSEFISFVTGEASDKCQKEKRKTINGDDLLWAMTTLGFEDYVEPLKVYLQRFREIEGE
RTGLGRPQTGGEVGEHQRDAVGDGGGFYGGGGMQYHQHHQFLHQQNHMYGATGGGSDSG
GGAASGRTRT*
```

cDNA Sequence

60/166,128

>G482 cDNA Sequence

TCGACCCACGCGTCCGGACACTTAACAATTCACACCTTCTCTTTTTACTCTTCCTAAAAC
CCTAAATTTCCTCGCTTCAGTCTTCCCACTCAAGTCAACCACCAATTGAATTCGATTTCG
AATCATTGATGGAAATGATTTGAAAAAAGAGTAAAGTTTATTTTTTTATTCCTTGTAATT
TTCAGAAATGGGGGATTCCGACAGGGATTCCGGTGGAGGGCAAAACGGGAACAACCAGAA
CGGACAGTCCTCCTTGTCTCCAAGAGAGCAAGACAGGTTCTTGCCGATCGCTAACGTCAG
CCGGATCATGAAGAAGGCCTTGCCCGCCAACGCCAAGATCTCTAAAGATGCCAAAGAGAC
GATGCAGGAGTGTGTCTCCGAGTTCATCAGCTTCGTCACCGGAGAAGCATCTGATAAGTG
TCAGAAGGAGAAGAGGAAGACGATCAACGGAGACGATTTGCTCTGGGCTATGACTACTCT
AGGTTTTGAGGATTATGTTGAGCCATTGAAAGTTTACTTGCAGAGGTTTAGGGAGATCGA
AGGGGAGAGGACTGGACTAGGGAGGCCACAGACTGGTGGTGAGGTCGGAGAGCATCAGAG
AGATGCTGTCGGAGATGGCGGTGGGTTCTACGGTGGTGGTGGTGGGATGCAGTATCACCA
ACATCATCAGTTTCTTCACCAGCAGAACCATATGTATGGAGCCACAGGTGGCGGTAGCGA
CAGTGGAGGTGGAGCTGCCTCCGGTAGGACAAGGACTTAACAAAGATTGGTGAAGTGGAT
CTCTCTCTGTATATAGATACATAAATACATGTATACACATGCCTATTTTTACGACCCATA
TAAGGTATCTATCATGTGATAGAACGAACATTGGTGTTGGTGATGTAAAATCAGATGTGC
ATTAAGGGTTTAGATTTTGAGGCTGTGTAAAAGAAGATCAAGTGTGCTTTGTTGGACAAT
AGGATTCACTAACGAATCTGCTTCATTGGATCTTGTATGTAACTAAAGCCATTGTATTGA
ATGCAAATGTTTTCATTTGGGATGCTTTAAAAAAAAAAAAAAAAA

Genomic Sequence

>G482 Genomic Sequence

ATGGGGGATTCCGACAGGGATTCCGGTGGAGGGCAAAACGGGAACAACCAGAACGGACAG
TCCTCCTTGTCTCCAAGAGAGCAAGACAGGTTCTTGCCGATCGCTAACGTCAGCCGGATC
ATGAAGAAGGCCTTGCCCGCCAACGCCAAGATCTCTAAAGATGCCAAAGAGACGATGCAG
GAGTGTGTCTCCGAGTTCATCAGCTTCGTCACCGGAGAAGCATCTGATAAGTGTCAGAAG
GAGAAGAGGAAGACGATCAACGGAGACGATTTGCTCTGGGCTATGACTACTCTAGGTTTT
GAGGATTATGTTGAGCCATTGAAAGTTTACTTGCAGAGGTTTAGGGAGATCGAAGGGGAG
AGGACTGGACTAGGGAGGCCACAGACTGGTGGTGAGGTCGGAGAGCATCAGAGAGATGCT
GTCGGAGATGGCGGTGGGTTCTACGGTGGTGGTGGTGGGATGCAGTATCACCAACATCAT
CAGTTTCTTCACCAGCAGAACCATATGTATGGAGCCACAGGTGGCGGTAGCGACAGTGGA
GGTGGAGCTGCCTCCGGTAGGACAAGGACTTAA

Alternatively, a fragment of a sequence from Table 1 is ^{32}P-radiolabeled by random priming (Sambrook et al., (1989) *Molecular Cloning. A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, New York) and used to screen a plant genomic library. As an example, total plant DNA from *Arabidopsis thaliana*, Nicotiana *tabacum*, *Lycopersicon pimpinellifolium*, Prunus *avium*, Prunus *cerasus*, Cucumis *sativus*, or *Oryza sativa* are isolated according to Stockinger al (Stockinger, E. J., et al., (1996), *J. Heredity*, 87:214-218). Approximately 2 to 10 µg of each DNA sample are restriction digested, transferred to nylon membrane (Micron Separations, Westboro, MA) and hybridized. Hybridization conditions are: 42° C in 50% formamide, 5X SSC, 20 mM phosphate buffer 1X Denhardt's, 10% dextran sulfate, and 100µg/ml herring sperm DNA. Four low stringency washes at RT in 2X SSC, 0.05% sodium sarcosyl and 0.02% sodium pyrophosphate are performed prior to high stringency washes at 55° C in 0.2X SSC, 0.05% sodium sarcosyl and 0.01% sodium pyrophosphate. High stringency washes are performed until no counts are detected in the washout according to Walling et al. (Walling, L. L., et al., (1988) Nucl. Acids Res. 16:10477-10492).

All references (publications and patents) are incorporated herein by reference in their entirety for all purposes.

Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

preferably at least 50%, 60%, 70% or 80% sequence identity, and more preferably 85%, 90%, 95% or 97% sequence identity. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein.

One way to identify whether two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions. Generally, stringent conditions are selected to be about 5°C to 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Conditions for nucleic acid hybridization and calculation of stringencies can be found in Sambrook et al. (1989) *Molecular Cloning. A Laboratory Manual*, Ed. 2, Cold Spring Harbor Laboratory Press, New York and Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* Part I, Elsevier, New York . Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For detecting less closely related homologs washes may be performed at 50° C.

For conventional hybridization the hybridization probe is conjugated with a detectable label such as a radioactive label, and the probe is preferably of at least 20 nucleotides in length. As is well known in the art, increasing the length of hybridization probes tends to give enhanced specificity. The labeled probe derived from the *Arabidopsis* nucleotide sequence may be hybridized to a plant cDNA or genomic library and the hybridization signal detected using means known in the art. The hybridizing colony or plaque (depending on the type of library used) is then purified and the cloned sequence contained in that colony or plaque isolated and characterized. Homologs may also be identified by PCR-based techniques, such as inverse PCR or RACE, using degenerate primers. See Ausubel et al. (eds) (1998) *Current Protocols in Molecular Biology*, John Wiley & Sons.

TF homologs may alternatively be obtained by immunoscreening an expression library. With the provision herein of the disclosed TF nucleic acid sequences, the polypeptide may be expressed and purified in a heterologous